



PROTOCOL TO IMAC PROTEIN PURIFICATION

1. Flow ethanol
2. Wash column with 3 volumes of double distilled water
3. Recharge column with 1 volume of 100 mM nickel sulphate
4. Wash 3 volumes of double distilled water
5. Equilibrate with 5 volumes of lysis buffer without imidazole
6. Flows lysate of step 8 of “Protocol to production of [6xHis]-GFP” and recover flow through fraction (FT) in a falcon
7. Wash with 3 volumes of lysis buffer without imidazole and recover wash fractions (W1-W3).
8. Flow gradient and recover correspondent fractions.
9. Wash with 3 volumes of double distilled water and store with Ethanol 20%
10. Run a SDS-PAGE of each fraction and keep that of interest.
11. Sample dialysis against 2L of phosphate buffer, change buffer after 1h at 4°C under weak agitation, and after overnight dialysis against same conditions.
12. Keep protein in ice until use.
13. Wash dialysis membrane with double distilled water and keep with sodium azide

GRADIENT

10 mM (A e B)

4,8 mL of Lysis buffer without imidazole

0,2 mL of Lysis buffer with 250mM imidazole

25 mM (A e B)

4,5 mL of Lysis buffer without imidazole

0,5 mL of Lysis buffer with 250mM imidazole

50 mM (A e B)

4 mL of Lysis buffer without imidazole

1 mL of Lysis buffer with 250mM imidazole

75 mM (A e B)

3,5 mL of Lysis buffer without imidazole

1,5 mL of Lysis buffer with 250mM imidazole

100 mM (A e B)

3 mL of Lysis buffer without imidazole

2 mL of Lysis buffer with 250mM imidazole

250 mM (A , B e C)

5,0 mL of Lysis buffer with 250mM imidazole

Buffer Recipes

Lysis buffer (1L):

Tris – 1,21g

NaH₂PO₄ – 6,89g

NaCl – 5,84g

Adjust pH to 8,0 and complete volume to 1L. Autoclave at 121°C for 15 min.

Store at 4°C.

Lysis buffer with 250 mM Imidazole (1L):

Tris – 1,21g

NaH₂PO₄ – 6,89g

NaCl – 5,84g

Imidazole – 17,02g

Adjust pH to 8,0 and complete volume to 1L. Autoclave at 121°C for 15 min.

Store at room temperature.

Phosphate buffer 10X (1L):

KCl – 2,0g

NaH₂PO₄ – 14,4g

NaCl – 80,0g

KH₂PO₄ – 2,4g

Adjust pH to 7,4 and complete volume to 1L. Autoclave at 121°C for 15 min.

Store at room temperature.